# SUMMARY OF SAFETY AND EFFECTIVENESS DATA

## 1. General Information

1.1. Device Generic Name: Immunoassay for antibody to hepatitis B surface antigen

1.2. Device Trade Name: ETI-AB-AUK PLUS

1.3. Applicant's Name and Address:

DiaSorin
Via Crescentino
Saluggia (VC) 13040, Italy

1.4. U.S. Representative:

Sienna Partners, LLC
P.O. Box 103
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1.5. PMA Number: P990042

1.6. Date of Panel Recommendation: January 20, 2000

1.7. Date of Notice of Approval to the Applicant: March 30, 2001

## 2. Indications For Use

ETI-AB-AUK PLUS is an in vitro enzyme immunoassay (EIA) intended for the qualitative detection of antibodies to hepatitis B surface antigen (anti-HBs) in human serum or plasma (EDTA, citrate or heparin). The ETI-AB-AUK PLUS is intended for manual use only.

The detection of anti-HBs is indicative of laboratory diagnosis for seroconversion from hepatitis B virus (HBV) infection. Anti-HBs is also used to assess laboratory diagnosis of past exposure to hepatitis B in potential hepatitis B vaccine recipients and to determine the presence of an immune response in vaccine recipients. The anti-HBs assay's performance has not been established for the monitoring of HBV disease or therapy.

## 3. Device Description

3.1. Principle of the Assay

The assay is a direct, non-competitive enzyme immunoassay based on the use of polystyrene microwells coated with recombinant HBsAg (subtypes ad and ay). An enzyme tracer containing horseradish peroxidase-labeled HBsAg (human, subtypes ad and ay) detects any captured anti-HBs from the patient's specimen.

In the assay procedure, patient specimens and controls are incubated with incubation buffer in hepatitis B surface antigen-coated microwells. If anti-HBs is present in the sample, it binds to the antigen. Excess sample is removed by a wash step, and the enzyme tracer is

then added to the microwells and allowed to incubate. The enzyme tracer binds to any antigen-antibody complexes present in the microwells. Excess enzyme tracer is removed by a wash step, and a chromogen/substrate solution is added to the microwells and allowed to incubate. If a sample contains anti-HBs, the bound enzyme (horseradish peroxidase) chemically reduces the substrate peroxide, which concurrently oxidizes the chromogen tetramethylbenzidine (TMB) to a blue color (650 nm). The blue color turns to yellow (450 nm) after addition of the stop solution. If a sample does not contain anti-HBs, the microwell will be colorless after the chromogen/substrate solution is added and will remain colorless after the stop solution is added. Color intensity, which is measured spectrophotometrically, is indicative of the concentration of anti-HBs. Absorbance value readings for patient specimens are compared to a cutoff value determined from the mean absorbance of the calibrator.

3.2. Kit Configuration and Components

For detection of total anti-HBs, the ETI-AB-AUK PLUS system is comprised of the following:

Coated Strips
Microwells coated with recombinant HBsAg (subtypes ad and ay, molecular weights 23 Kd, 46 Kd and 69 Kd, expressed in Hansenula polymorpha).

Enzyme Tracer
Horseradish peroxidase-labeled human HBsAg (subtypes ad and ay), buffer, protein stabilizers.
Preservative: 0.2% ProClin 300.

Tracer Diluent
Human serum/plasma, buffer.
Preservative: 0.2% ProClin 300.

Immunity Calibrator
Human serum/plasma containing 15 mIU/mL anti-HBs referenced to WHO Anti-Hepatitis B Immunoglobulin International Reference Preparation, protein stabilizers.
Preservative: 0.2% ProClin 300.

Negative Control (Human)
Human serum/plasma non-reactive for all known HBV markers.
Preservative: 0.2% ProClin 300.

Positive Control (Human)
Human serum/plasma reactive for anti-HBs, protein stabilizers.
Preservative: 0.2% ProClin 300.

Incubation Buffer
Buffer, protein stabilizers, an inert blue dye.
Preservative: 0.2% ProClin 300.

Wash Buffer (concentrate)
Buffer, detergents, preservatives.

Chromogen/Substrate
Tetramethylbenzidine/hydrogen peroxide system.

Stop Solution
1N sulfuric acid

Strip Sealers

Plate Sealers

Pouch Sealers

## 4. Contraindications

None

## 5. Warnings and Precautions

For in vitro diagnostic use only.

Warnings and precautions for users of the ETI-AB-AUK PLUS assay are stated in the product labeling.

## 6. Alternative Practices and Procedures

At this time several methods are available for detecting serologic anti-HBs in human serum or plasma. Methods commonly used include passive hemagglutination of antigen-sensitized red blood cells, radioimmunoassays, and enzyme immunoassays.

## 7. Marketing History

The ETI-AB-AUK PLUS assay has never been marketed in the US or outside the US.

## 8. Potential Adverse Effects of the Device on Health

A false positive result may be considered a patient or public health concern because of the fact that the patient may be considered in seroconversion and proceeding to HBV recovery. In this case, the risk may be that the patient's treatment may be altered or that they might not be considered infectious. This could lead to possible exposure to other uninfected individuals. In the situation of pre-vaccination testing, a false positive result could lead to no vaccination and, consequently, a higher risk of infection if exposed to HBV and of spreading to non-immune individuals. In the situation of post-vaccination, a false positive result could lead to the conclusion that the individual had reached the immunity threshold when they had not.

## 9. Summary of Preclinical Studies

### 9.1. Potential Cross-Reacting Substances

Serum samples were obtained from patients belonging to number of different disease catergories listed below. Of the 535 potentially interfering samples, 450 (84%) were negative and 85 (16%) were positive by ETI-AB-AUK PLUS. Among the 85 positive samples, 82 were positive by reference testing. Percent positive was 96% (82/85). Due to the expression of the recombinant HBsAg in yeast, samples containing antibodies to yeast may cause false positive results. In this study, two out of ten (20%) patients with yeast infections gave false positive results. Disease was determined by serological testing, there

is no guarantee that the associated antibody was present in the tested material. Interference testing with the described specimens was not performed.

Cross-Reactivity Study Results

| GROUP | n | ETI-AB-AUK PLUS Negative or Equivocal Samples | ETI-AB-AUK PLUS Positive Samples | % Positive By Additional Testing |
|---|---|---|---|---|
| Acute EBV infection | 16 | 10 | 6 | 83% (5/6) |
| Acute CMV infection | 20 | 19 | 1 | 100% (1/1) |
| Acute HSV infection | 10 | 10 | 0 | – |
| Acute toxoplasmosis | 18 | 16[a] | 2 | 100% (2/2) |
| Acute parvovirus B19 infection | 5 | 4 | 1 | 100% (1/1) |
| HTLV-I/II infection | 50 | 43 | 7 | 100% (7/7) |
| Syphilis | 26 | 22[b] | 4 | 75% (3/4) |
| HCV infection | 50 | 45 | 5 | 100% (5/5) |
| HDV infection | 20 | 20 | 0 | – |
| HIV infection | 50 | 42 | 8 | 100% (8/8) |
| Acute HAV infection | 50 | 45 | 5 | 100% (5/5) |
| Past HAV infection | 50 | 38 | 12 | 92% (11/12)[c] |
| Rheumatoid factor (RF) + | 40 | 37 | 3 | 100% (3/3) |
| Autoimmune disease, including SLE | 30 | 29 | 1 | 100% (1/1) |
| Autoimmune hepatitis | 5 | 5 | 0 | – |
| Myeloma | 20 | 14 | 6 | 100% (6/6) |
| Hypergammaglobulinemia | 20 | 17 | 3 | 100% (3/3) |
| Influenza vaccine | 5 | 4 | 1 | 100% (1/1) |
| Elevated liver enzymes | 10 | 7 | 3 | 100% (3/3) |
| Cirrhosis | 20 | 16 | 4 | 100% (4/4) |
| Alcoholic hepatitis | 10 | 5 | 5 | 100% (5/5) |
| Yeast infection | 10 | 2 | 8 | 75% (6/8) |
| TOTAL | 535 | 450 (84%) | 85 (16%) | 96% (82/85) |

[a] 1 sample was DiaSorin repeatedly equivocal.
[b] 1 sample was DiaSorin equivocal, not repeated.
[c] 1 sample was total anti-HBc and anti-HBe positive, indicating recovery with Abbott anti-HBs result false negative.

9.2. Interfering Substances

The ETI-AB-AUK PLUS assay was evaluated for interference by testing the following substances listed below. Testing was performed using matched pairs of negative donor serum and negative donor serum spiked with high-titer anti-HBs samples to obtain a result near the cutoff. None of the compounds at the levels indicated below were found to interfere with the clinical interpretation of the assay in serum. No interference was found with bilirubin in plasma (EDTA, heparin or citrate), testing for interference with hemoglobin and triolein was not performed in plasma.

| Compound | Concentration | |
|---|---|---|
| Bilirubin | 0.35 mmol/L | 20 mg/dL |
| Hemoglobin | 0.06 mmol/L | 100 mg/dL |
| Triolein | 33.9 mmol/L | 3000 mg/dL |

9.3. Stability Studies

9.3.1. Kit Stability

Stability studies were performed on 3 different ETI-AB-AUK PLUS kit lots. At specified intervals from time of kit release, performance of the kits was evaluated testing the Calibrator, Negative and Positive Controls, total anti-HBs calibration curve (World Health Organization [WHO] referenced), and Q.C. sera panel according to the instructions for use. The kit must meet established acceptance criteria. The obtained stability data demonstrate that the kit performance is acceptable for at least 13 months. On the basis of the stability results, a shelf life of 13 months has been established for the kit.

9.3.2. Working Enzyme Tracer Stability

The Enzyme Tracer was diluted with the Tracer Diluent to obtain the working Enzyme Tracer according to the instructions for use. After 7 days from dilution, the performance of the kit was evaluated, according to the instructions for use, testing various specimens with freshly prepared working Enzyme Tracer and the 7-days old working Enzyme Tracer. The kit must meet established acceptance criteria. The tests on the working Enzyme Tracer demonstrate that the performance of the kit is acceptable when the 7 day-diluted Enzyme Tracer is used. The working Enzyme Tracer can be used for one week if stored at 2-8 °C.

9.3.3. Working Wash Buffer Stability

The Wash Buffer concentrate was diluted with deionized water according to the instruction for use to obtain the working Wash buffer. After 7 days from dilution, the performance of the kit was evaluated by testing various specimens with a freshly prepared working Wash buffer and the 7-days-old working Wash buffer, according to the instructions for use. The kit must meet established acceptance criteria. The tests

on the working Wash Buffer demonstrate that the performance of the kit is acceptable when the 7 day-old working Wash Buffer is used. The working Wash Buffer can be used for one week if stored at 2-8 °C.

9.4. Common Reagents Interchangeablity Study

Studies were performed to demonstrate that the lots of some components included with ETI-AB-AUK PLUS kit and common to all kits of ETI-PLUS line (Wash Buffer, Chromogen/Substrate, Stop Solution), can be exchanged with other lots of the same component produced for the ETI-PLUS line (interchangeablity). Three lots of Wash Buffer, Chromogen/Substrate and Stop Solution were combined with one lot of ETI-AB-AUK PLUS; the three combinations were then tested with various samples. Regression analysis was applied to the Optical Density of the samples. The regression analyses for the three studies gave slopes close to 1.0, with low intercepts and excellent correlation values. These results indicate that the use of different lots of Wash Buffer, Chromogen/Substrate and Stop Solution with the same ETI-AB-AUK PLUS lot gave equivalent results with samples distributed over the range of reactivity, confirming their interchangeablity.

9.5. Reproducibility

Manual Assay: Intra-assay, inter-assay, inter-lot, and inter-site variability studies were carried out on the ETI-AB-AUK PLUS kit to test the variability within runs, between runs, between days, between kit lots, and between test sites. Variability was measured on a panel of ten sera that included negative, borderline, and positive samples. Three ETI-AB-AUK PLUS kit lots were tested at three independent test sites. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance to cutoff ratios (S/CO) rather than absolute absorbance values. The results of that study are tabulated below showing the reproducibility of the assay to be satisfactory

Clinical Site Serum Reproducibility Study

| ID# | | # of Tests per Sample | Mean S/CO' | Within-run %CV* | Between-runs %CV | Between-lots %CV | Between-days %CV | Between-sites %CV | Total |
|---|---|---|---|---|---|---|---|---|---|
| S01 | High | 108 | 7.55 | 7.35 | 7.62 | 5.76 | 1.81 | 26.33 | 10.04 |
| S02 | High | 108 | 4.03 | 3.69 | 6.22 | 5.08 | 3.64 | 21.60 | 12.67 |
| S03 | Low | 108 | 2.26 | 4.80 | 6.59 | 7.57 | 3.76 | 44.20 | 13.29 |
| S04 | Equiv | 108 | 1.11 | 7.98 | 5.28 | 7.96 | 3.95 | 22.29 | 13.45 |
| S05 | Equiv | 108 | 1.22 | 7.23 | 7.20 | 8.58 | 3.29 | 17.56 | 14.49 |
| S06 | Equiv | 108 | 0.81 | 3.55 | 7.99 | 6.56 | 3.09 | 26.25 | 16.94 |
| S07 | Equiv | 108 | 0.54 | 5.01 | 6.66 | 11.54 | 1.68 | 31.66 | 11.38 |
| S08 | Neg | 108 | 0.31 | 10.63 | 9.87 | 10.87 | 4.78 | 16.26 | 18.21 |
| S09 | Neg | 108 | 0.24 | 9.69 | 8.57 | 14.18 | 7.87 | 15.51 | 24.79 |
| S10 | Neg | 108 | 0.03 | 12.86 | 25.65 | 54.94 | 55.23 | 112.01 | 125.64 |

* %CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate

No reproducibility testing with plasma was conducted. As part of the conditions of approval agreement, DiaSorin will provide FDA results from a reproducibility study using EDTA, heparin and citrate plasma samples. Until that condition is met, the following statement will be placed in the labeling:

"Assay reproducibility using plasma has not been established with samples near the cutoff. If plasma is used (EDTA, citrate or heparin), the user should establish appropriate assay reproducibility in accordance with NCCLS EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices."

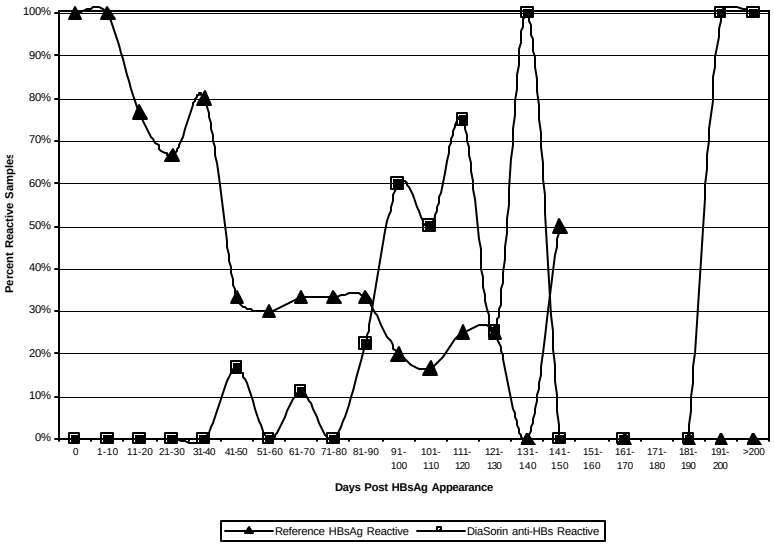
9.6. Acute Serial Seroconversion Panels

One hundred twenty-four (124) archived serial samples from nine individuals were tested to determine when anti-HBs antibodies appear in relation to HBsAg. Most (8/9) of these individuals were defined as being acutely infected by the appearance of HBsAg and HBeAg with the subsequent appearance of IgM anti-HBc, total anti-HBc, anti-HBe, and anti-HBs. One individual had detectable HBsAg, but did not have detectable HBeAg in any specimen. However, this individual did seroconvert for anti-HBe.

The specimens were collected from individuals undergoing plasmapheresis for further manufacturing purposes. Three individuals were found to be infected with HBV during the first plasmapheresis and others became infected with HBV during subsequent plasmapheresis. It is unknown how long these three initially HBsAg reactives were infected prior to the first plasmapheresis. All nine individuals underwent sequential plasmapheresis after becoming HBV infected. However, the timing of subsequent plasmapheresis varied from individual to individual. The specimens draw times were normalized to represent the day that HBsAg was first detected by an FDA-licensed assay as day 0. Draw days ranged from day 0 (HBsAg first detected) through day 355 post-day 0. Since all panels did not contain the same draw day, sample results were grouped within day intervals (e.g., days 0, 1-10, 11-20, etc., representing days since first detection of HBsAg). The earliest anti-HBs was detected was 81-90 days after the detection of HBsAg.

The results are summarized in the following table and graph. In the graph below, the graph for the reference HBsAg percent reactive has been overlaid for reference.

| Day Range | Number Specimens | DiaSorin anti-HBs Reactive | % Positive |
|---|---|---|---|
| 0 | 9 | 0 | 0.0% |
| 1-10 | 10 | 0 | 0.0% |
| 11-20 | 13 | 0 | 0.0% |
| 21-30 | 9 | 0 | 0.0% |
| 31-40 | 10 | 0 | 0.0% |
| 41-50 | 6 | 0 | 0.0% |
| 51-60 | 10 | 0 | 0.0% |
| 61-70 | 9 | 0 | 0.0% |
| 71-80 | 6 | 0 | 0.0% |
| 81-90 | 9 | 2 | 22.2% |
| 91-100 | 10 | 4 | 40.0% |
| 101-110 | 6 | 1 | 16.7% |
| 111-120 | 4 | 0 | 0.0% |
| 121-130 | 4 | 0 | 0.0% |
| 131-140 | 3 | 3 | 100.0% |
| 141-150 | 2 | 0 | 0.0% |
| 151-160 | 0 | 0 | NA |
| 161-170 | 1 | 0 | 0.0% |
| 171-180 | 0 | 0 | NA |
| 181-190 | 1 | 0 | 0.0% |
| 191-200 | 1 | 0 | 0.0% |
| 355 | 1 | 1 | 100.0% |




9.7. High Risk Population

Single repository samples belonging to high-risk populations (66 hemodialyzed patients, 148 hemophiliacs, 150 IV drug users) were tested with the DiaSorin ETI-AB-AUK PLUS assay to determine frequency of positive results in that population. The group was 12% (42/364) female, 69% (252/364) male, and 19% (70/364) unspecified, with ages ranging from 19 to 87 years old. No geographical locations were specified. The table below summarizes the ETI-AB-AUK PLUS results. The data in the table represent the number of specimens in each category.

High Risk Population

| Population | Frequency of Positive Results (# Positive/Total # Samples) |
|---|---|
| IV drug users | 36/150 = 24.0% (3 equivocal) |
| Hemophiliacs | 37/148 = 25.0% (1 equivocal) |
| Hemodialysis patients | 21/66 = 31.8% (2 equivocal) |
| TOTAL | 94/364 = 25.8% (6 equivocal) |

9.8. Expected Values Study

The 236 prospective samples used in the expected values study for detecting anti-HBs by the ETI-AB-AUK PLUS assay were from patients who were sent to the laboratory for HBV testing. Of those, 100 (42%) were frozen and 136 (58%) were fresh. The patients represented Florida, Georgia, Pennsylvania, California, Utah, and the southeastern US. The group was 69% (162/236) female, 29% (68/236) male, and 2% (6/236) unspecified; the ethnicity of the patients was unspecified. The ages ranged from 5 to 88 years old, with 6 samples unspecified. The prevalence rate for anti-HBs in patients who were sent to the laboratory for HBV testing was 25%.

The table below summarizes the percent DiaSorin ETI-AB-AUK PLUS positive and negative results by gender and age range. There were 6 samples for which gender and age were not reported; they were all negative. There were 6 other samples for which age was not reported, 2 were from females and 4 were from males; 2 were positive and 4 were negative. These 12 results were not included in the table. DiaSorin equivocal results were not repeated in this study.

Expected Values Summary

| | | DiaSorin ETI-AB-AUK PLUS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | + | | - | | E* | | TOTAL |
| Age Range | Gender | N | % | n | % | n | % | |
| 0-9 | F | 0 | 0% | 2 | 100% | 0 | 0% | 2 |
| | M | 0 | 0% | 0 | 0% | 0 | 0% | 0 |
| 10-19 | F | 3 | 18% | 14 | 82% | 0 | 0% | 17 |
| | M | 1 | 50% | 1 | 50% | 0 | 0% | 2 |
| 20-29 | F | 8 | 16% | 43 | 84% | 0 | 0% | 51 |
| | M | 4 | 31% | 9 | 69% | 0 | 0% | 13 |
| 30-39 | F | 8 | 16% | 40 | 82% | 1 | 2% | 49 |
| | M | 5 | 29% | 11 | 65% | 1 | 6% | 17 |
| 40-49 | F | 7 | 35% | 13 | 65% | 0 | 0% | 20 |
| | M | 5 | 36% | 9 | 64% | 0 | 0% | 14 |
| 50-59 | F | 4 | 80% | 1 | 20% | 0 | 0% | 5 |
| | M | 4 | 50% | 4 | 50% | 0 | 0% | 8 |
| 60-69 | F | 0 | 0% | 3 | 100% | 0 | 0% | 3 |
| | M | 0 | 0% | 2 | 100% | 0 | 0% | 2 |
| 70-79 | F | 3 | 30% | 7 | 70% | 0 | 0% | 10 |
| | M | 3 | 60% | 2 | 40% | 0 | 0% | 5 |
| 80-89 | F | 0 | 0% | 3 | 100% | 0 | 0% | 3 |
| | M | 0 | 0% | 3 | 67% | 0 | 0% | 3 |
| TOTAL | | 55 | 25% | 166 | 74% | 2 | 1% | 224 |

*E = equivocal result

## 10. Summary of Clinical Studies

### 10.1. Clinical Sample Testing

#### 10.1.1. Prospective Samples

A study of 136 prospective specimens was conducted. These specimens represented individuals who were sent to the laboratory for hepatitis testing. Specimens were collected at a reference laboratory and assayed at the California clinical trial site. The patients were 86% (117/136) female and 14% (19/136) male. The ages ranged from 5 to 77 years old, with three specimens not specified.

The study (testing) sites were blinded to the previous specimen categorization. All testing was performed by the manual ETI-AB-AUK PLUS procedure. Specimens were characterized by testing with six HBV serological markers (HBsAg, HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, anti-HBs) using FDA-licensed or approved assays. Testing with these assays followed the FDA-licensed or approved labeling procedure, including confirmation by neutralization of repeatably reactive HBsAg samples.

Results by Specimen Classification

After study completion all samples were assigned a specimen classification based on the patterns of the six HBV serological markers established by the reference assays. Based on these serological marker patterns, the samples were categorized into the HBV classifications described in the table below. There were 5 unique HBV marker patterns observed in the ETI-AB-AUK PLUS prospective clinical studies.

| Characterization Based On Single Point Specimen | HBsAg | HBeAg | IgM anti-HBc | Total anti-HBc | anti-HBe | anti-HBs | n |
|---|---|---|---|---|---|---|---|
| Chronic Infection | + | - | - | + | + | - | 1 |
| Recovery | - | - | - | + | + | + | 2 |
| Past Infection | - | - | - | + | - | + | 4 |
| | - | - | - | + | - | - | 4 |
| HBV Vaccine Response | - | - | - | - | - | + | 38 |
| Not Previously Infected with HBV | - | - | - | - | - | - | 87 |

Based on the above classifications the ETI-AB-AUK PLUS anti-HBs results for the prospective samples were compared to a reference assay's anti-HBs results. The reference assay was not calibrated to the WHO standard of 10 mIU/mL established for immunity. The following table shows this comparison and percent agreement with 95% exact confidence intervals with the reference anti-HBs results. DiaSorin equivocal results were not repeated in this study.

Prospective Samples Comparison

| Reference Serology Classification | Reference Assay | | | | TOTAL |
|---|---|---|---|---|---|
| | - | + | | | |
| | ETI-AB-AUK PLUS | ETI-AB-AUK PLUS | | | |
| | - | - | + | E* | |
| Chronic infection | 1 | 0 | 0 | 0 | 1 |
| Recovery | 0 | 0 | 2 | 0 | 2 |
| Past infection | 4 | 0 | 4 | 0 | 8 |
| HBV vaccine response | 0 | 2 | 34 | 2 | 38 |
| Not previously infected | 87 | 0 | 0 | 0 | 87 |
| TOTAL | 92 | 2 | 40 | 2 | 136 |

*E = equivocal result

Prospective Samples Agreement Rates

| | | |
|---|---|---|
| Chronic Infection | Positive agreement with reference assay results = NA | (0/0) |
| | 95% CI = NA | |
| | Negative agreement with reference assay results = 100% | (1/1) |
| | 95% CI = 2.5 to 100% | |
| Recovery | Positive agreement with reference assay results = 100% | (2/2) |
| | 95% CI = 15.8 to 100% | |
| | Negative agreement with reference assay results = NA | (0/0) |
| | 95% CI = NA | |
| HBV Vaccine Response | Positive agreement with reference assay results = 89.5% | (34/38) |
| | 95% CI = 75.2 to 97.1% | |
| | Negative agreement with reference assay results = NA | (0/0) |
| | 95% CI = NA | |
| Past Infection | Positive agreement with reference assay results = 100% | (4/4) |
| | 95% CI = 39.8 to 100% | |
| | Negative agreement with reference assay results = 100% | (4/4) |
| | 95% CI = 39.8 to 100% | |
| Not Previously Infected | Positive agreement with reference assay results = NA | (0/0) |
| | 95% CI = NA | |
| | Negative agreement with reference assay results = 100% | (87/87) |
| | 95% CI = 98.5 to 100% | |

10.1.2. Retrospective Samples

Retrospective studies were carried out at three clinical laboratories in the United States (California, Missouri, and Minnesota) and at DiaSorin (Italy) to assess the performance of the ETI-AB-AUK PLUS assay in detecting anti-HBs. The study set included 650 frozen repository samples (the majority of which were purchased from commercial vendors) from the following populations:

- patients with chronic hepatitis B infection (HBsAg positive for greater than six months) - 111 frozen repository samples;

- patients with serologically diagnosed hepatitis B infection (acute, chronic, asymptomatic, convalescent, etc.) - 82 frozen repository samples;
- patients sent to the laboratory for hepatitis B testing - 100 frozen repository samples;
- a general hospital patient population - 357 frozen repository samples.

The specimens represented Midwestern (2%), Southeastern (25%), Western (13%), and Northeastern US (2%), outside of the US (1%), and unspecified (57%). The group was 44% (287/650) female, 42% (270/650) male, and 14% (93/650) unspecified. Approximately 13% (84/650) were Caucasian, 4% (27/650) were African American, < 1% (5/650) were Hispanic, < 1% (3/650) were Asian, and 82% (531/650) were unspecified. The ages ranged from 5 to 98 years old, with 131 specimens not specified.

The study (testing) sites were blinded to the previous specimen categorization. All testing was performed by the manual ETI-AB-AUK procedure. Specimens were characterized by testing with six HBV serological markers (HBsAg, HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, anti-HBs) using FDA-licensed or approved assays. Testing with these assays followed the FDA-licensed or approved procedure with the exception of the HBsAg assay at two of the three sites. At these sites, the majority of specimens that were initially HBsAg-positive were repeated in duplicate, however the repeatedly reactive specimens were not confirmed by the licensed HBsAg confirmation assay at the two sites. Therefore, true HBsAg result was determined in one of three ways: 1) confirmed by reference assay neutralization during clinical trials, 2) based on a statement by the attending physician that HBsAg was positive for greater than 6 months, or 3) information provided by the vendor regarding confirmatory testing performed at their location or by the material source facility.

Results by Specimen Classification

After study completion all samples were assigned a specimen classification based on the patterns of the six HBV serological markers established by the reference assays. Based on these serological marker patterns, the samples were categorized into the HBV classifications described in the table below. There were 35 unique HBV marker patterns observed in the ETI-AB-AUK PLUS retrospective clinical studies.

| Characterization Based On Single Point Specimen | HbsAg | HBeAg | IgM anti-HBc | Total anti-HBc | anti-HBe | anti-HBs | n |
|---|---|---|---|---|---|---|---|
| Acute Infection | + | + | + or I* | + | - | - | 52 |
| | + | - | + or I | + | + | - | 4 |
| | + | - | - | - | - | - | 2 |
| | + | + | - | - | - | - | 2 |
| Chronic Infection | + | - | - | + | + | - | 82 |
| | + | + | - | + | - | - | 21 |
| | + | - | - | + | - or I | - | 23 |
| | + | + | + | + | - | + | 4 |
| | + | + | - or I | + | - | + | 2 |
| | + | - | - | + | + | + | 2 |
| | + | + | - | + | + or I | + | 2 |
| | + | + | + | + | + | + | 1 |
| | + | + | - | + | + | - | 1 |
| | + | - | - | + | - | + | 1 |
| Recovery | - | - | - | + | + or I | + | 40 |
| | - | - | - | + | + | - | 6 |
| | - | - | + | + | + | - | 2 |
| | - | - | + or I | + | + | + | 2 |
| Past Infection | - | - or I | - | + | - | + | 12 |
| | - | - | - | + | - | - | 9 |
| HBV Vaccine Response | - | - | - | - | - | + | 20 |
| Not Previously Infected with HBV | - | - | - | - | - | - | 343 |
| Uninterpretable | - | + or I | - | - | - | - | 13 |
| | - | + | - | + | - | + | 2 |
| | - | + | - | + | + | + | 1 |
| | - | I | - | + | - | - | 1 |

*I = indeterminate result

Based on the above classifications the ETI-AB-AUK PLUS results for the retrospective samples were compared to a reference assay's anti-HBs results. The reference assay was not calibrated to the WHO standard of 10 mIU/mL established for immunity. The following table shows this comparison and percent agreement with 95% confidence intervals with the reference anti-HBs results. DiaSorin equivocal results were not repeated in this study.

Retrospective Samples Comparison

| Reference Serology Classification | Reference Assay | | | | | TOTAL |
|---|---|---|---|---|---|---|
| | - | | + | | | |
| | ETI-AB-AUK PLUS | | ETI-AB-AUK PLUS | | | |
| | - | + | - | + | E* | |
| Acute infection | 60 | 0 | 0 | 0 | 0 | 60 |
| Chronic infection | 127 | 0 | 8 | 3 | 1 | 139 |
| Recovery | 7 | 1 | 6 | 35 | 1 | 50 |
| Past infection | 9 | 0 | 4 | 8 | 0 | 21 |
| HBV vaccine response | 0 | 0 | 5 | 15 | 0 | 20 |
| Not previously infected | 341 | 2 | 0 | 0 | 0 | 343 |
| Uninterpretable | 14 | 0 | 2 | 1 | 0 | 17 |
| TOTAL | 558 | 3 | 25 | 62 | 2 | 650 |

* E = equivocal result

Retrospective Samples Agreement Rate

Acute Infection

Positive agreement with reference assay results = NA (0/0)
95% CI = NA

Negative agreement with reference assay results = 100% (60/60)
95% CI = 90.0 – 100%

Chronic Infection

Positive agreement with reference assay results = 25.0% (3/12)
95% CI = 5.5 – 57.2%

Negative agreement with reference assay results = 100% (127/127)
95% CI = 97.1 – 100%

Recovery

Positive agreement with reference assay results = 83.3% (35/42)
95% CI = 68.6% – 93.0%

Negative agreement with reference assay results = 87.5% (7/8)
95% CI = 47.3 – 99.7%

Past Infection

Positive agreement with reference assay results = 66.7% (8/12)
95% CI = 34.9 – 90.1%

Negative agreement with reference assay results = 100% (9/9)
95% CI = 66.4 – 100%

HBV Vaccine Response

Positive agreement with reference assay results = 75.0% (15/20)
95% CI = 50.9 to 91.3%

Negative agreement with reference assay results = NA (0/0)
95% CI = NA

Not Previously Infected

Positive agreement with reference assay results = NA (0/0)
95% CI = NA

Negative agreement with reference assay results = 99.4% (341/343)
95% CI = 97.9 to 99.9%

Uninterpretable

Positive agreement with reference assay results = 33.3% (1/3)
95% CI = 0.8 to 90.6%
Negative agreement with reference assay results = 100% (14/14)
95% CI = 76.8% - 100%

10.1.3 Clinical Performance with Individuals Who Have Received Hepatitis B Vaccine

A retrospective study was conducted to evaluate a total of 59 serum samples from subjects who had received a full course of injections (3) from either SmithKline-Beecham Biologicals Engerix-B® HBV vaccine or Merck & Co., Inc. Recombivax HB® vaccine (one 90 or one 180 day vaccination regiment). These subjects had no pre-vaccination sample tested to indicate immunity status before vaccination. Results were expressed as "Immune" or "Not Immune" corresponding to a reference assay quantitative result of > 10 mIU/mL or a DiaSorin result ≥ mean absorbance value of the calibrator.

Vaccine Subjects

| DiaSorin Anti-HBs Result | Reference Anti-HBs Result[a] | | |
|---|---|---|---|
| | I[b] | NI[b] | Total |
| Immune | 44 | 3 | 47 |
| Not Immune | 0 | 12 | 12 |
| Grand Total | 44 | 15 | 59 |

[a] Reference assay cutoff was not calibrated to 10 mIU/mL
[b] I = Immune; NI = Not Immune

% Agreement Table

| | % (n) | 95% Exact Confidence Interval |
|---|---|---|
| Immune % Agreement with Reference Method | 100.0 (44/44) | 92.0 –100.0% |
| Not Immune % Agreement with Reference Method | 80.0 (12/15) | 51.9 – 95.7% |

10.1.4. Clinical Performance with Matched Pre- and Post-Vaccination Samples

Pre- and post-vaccination samples from 31 subjects who had received a full course of vaccinations were tested with the DiaSorin ETI-AB-AUK PLUS assay and a reference method. The results are shown in the table below. Results were expressed as "Immune" or "Not Immune" corresponding to a reference assay quantitative result of > 10 mIU/mL for post-vaccination samples or a DiaSorin result ≥ mean absorbance value of the calibrator. Reference assay results for pre-vaccination samples were not quantitated. However, all samples except 1 had absorbance values near the negative control, a sample that is negative for anti-HBs.

Pre-Vaccination Panel

| DiaSorin Anti-HBs Result | Reference Anti-HBs Result[a] | | |
|---|---|---|---|
| | I[b] | NI[b] | Total |
| Immune | 0 | 0 | 0 |
| Not Immune | 1 | 30 | 31 |
| Grand Total | 1 | 30 | 31 |

% Agreement Table

| | % (n) | 95% Exact Confidence Interval |
|---|---|---|
| Immune % Agreement with Reference Method | 0.0% (0/1) | 0.0 –97.5% |
| Not Immune % Agreement with Reference Method | 100.0% (30/30) | 88.4 – 100.0% |

Post-Vaccination Panel

| DiaSorin Anti-HBs Result | Reference Anti-HBs Result | | |
|---|---|---|---|
| | I | NI | Total |
| Immune | 28 | 0 | 28 |
| Not Immune | 0 | 2 | 2 |
| E* | 0 | 1 | 1 |
| Grand Total | 28 | 3 | 31 |

*E = Equivocal, not retested in this study

% Agreement Table

| | % (n) | 95% Exact Confidence Interval |
|---|---|---|
| Immune % Agreement with Reference Method | 100.0% (28/28) | 87.7 –100.0% |
| Not Immune % Agreement with Reference Method | 66.7% (2/3) | 9.4 – 99.0% |

Combined Pre- and Post-Vaccination Panels

| DiaSorin Anti-HBs Result | Reference Anti-HBs Result | | |
|---|---|---|---|
| | I | NI | Total |
| Immune | 28 | 0 | 28 |
| Not Immune | 1 | 32 | 33 |
| E* | 0 | 1 | 1 |
| Grand Total | 29 | 33 | 62 |

*E = Equivocal, not retested in this study

% Agreement Table

| | % (n) | 95% Exact Confidence Interval |
|---|---|---|
| Overall Immune % Agreement with Reference Method | 96.6% (28/29) | 82.2 – 99.9% |
| Overall Not Immune % Agreement with Reference Method | 97.0 (32/33) | 84.2 – 99.9% |

**11. Conclusions Drawn from Studies**

The study data demonstrates that acceptable performance is obtained with the DiaSorin ETI-AB-AUK PLUS assay when testing specimens collected in serum and plasma. The DiaSorin assay shows acceptable within-run, between-run, between-day, site-to-site, and lot-to-lot reproducibility. The quality control procedures described in the package insert are appropriate to assure accurate assay performance. The data from this study show that the DiaSorin ETI-AB-AUK PLUS assay is safe and effective for its stated purpose when used as instructed in the package insert. The DiaSorin ETI-AB-AUK PLUS total anti-HBs assay can be stored up to 13 months at 2-8°C.

Benefit/Risk

The submitted studies have shown that the DiaSorin ETI-AB-AUK PLUS assay, when compared to reference laboratory procedures, has a similar ability to detect the presence of antibodies to HBsAg in specimens from individuals acutely and chronically infected with HBV. The rate of false positivity and false negativity are within acceptable limits compared to the reference assay. It has been shown that the device has no demonstrable cross-reactivity with viruses or organisms that may cause clinical hepatitis. Therefore, the devices should benefit the physician in the diagnosis of HBV associated and non-associated hepatitis. The devices will also aid in the identification of those individuals who should and should not be vaccinated with the HBV. Therefore it is reasonable to conclude that the benefits of use of the device for the target population outweigh the risk of illness or injury when used as indicated when used accordance with the directions for use.

## 12. Panel Recommendations

The Microbiology Advisory Panel met on January 20, 2000, to consider the safety and effectiveness of the ETI-AB-AUK PLUS assay. The advisory panel recommended approval subject to the following conditions.

- Conduct additional studies for the immunity claim by testing individuals immediately after receiving the complete series of three vaccinations with the hepatitis B virus vaccines and three to nine months later.
- Provide more data on acute/chronic infections in high-risk populations such as those individuals that are infected with HIV, sexually transmitted diseases, and those patients that are immunosuppressed.
- Collect more data on patients meeting the standard definition for chronicity, i.e., > six months of infection.

## 13. CDRH Decision

CDRH concurred with the Panel's recommendation. DiaSorin Inc. has provided some additional data to address some of the Panel's issues and those issues not fully resolved were addressed with labeling restrictions and the requirement of postapproval studies. The two postapproval studies were:

- Within 6 months of this approval, DiaSorin Inc. should submit a new reproducibility study using the your new immunity cutoff, i.e., 15 mIU/ml.

- To address the concerns made by the Panel regarding the retrospective nature of the clinical studies, within 2 years of this approval, DiaSorin Inc. should submit the results of an additional prospective clinical study. We suggest that this study involve individuals that may be considered representative of an U.S. population, i.e., similar prevalence of HBV disease and serotypes.

The applicant's manufacturing facility was found to be in compliance with the Quality Systems Regulation (21 CFR 820).

CDRH issued an approval order on March 30, 2001.

## 13. Approval Specifications

Directions for use: See Labeling

Conditions of Approval: CDRH Approval of this PMA is subject to full compliance with the conditions described in the approval order.